Article

Distribution and abundance of *Eimeria* species in commercial turkey flocks across Canada

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Abstract — Diversity and regional abundance of *Eimeria* species infecting Canadian commercial turkey flocks are largely unknown. To address this paucity of data regarding coccidiosis and its distribution in Canada, fecal samples from turkey flocks (N = 39) representing 27 commercial farms [ON (*n* = 20), SK (*n* = 2), BC (*n* = 3), AB (*n* = 1), NS (*n* = 1)] were screened for coccidia. Identification of all *Eimeria* species present in each sample was accomplished using a nested-polymerase chain reaction (PCR) assay targeting the mitochondrial cytochrome *C* oxidase subunit I gene. Most samples (33/39) were *Eimeria*-positive with 6 *Eimeria* species identified by the nested-PCR assay (1 to 6 species/sample, average 3.2); 4 samples (4/39, > 10% of samples) contained all 6 species. *Eimeria* species were common and distributed widely in Canadian commercial turkey flocks. Turkeys reared using in-feed medication or live vaccination for coccidiosis control had similar *Eimeria* species diversity within individual flocks. These preliminary observations highlight that coccidiosis remains a concern for Canadian turkey producers.

Résumé — Distribution et abondance d'espèces d'*Eimeria* infectant des troupeaux de dindes commerciaux à travers le Canada. La diversité et l'abondance régionale d'espèces d'*Eimeria* infectants des troupeaux de dindes commerciaux canadiens sont pour la plupart inconnues. Pour adresser cette pénurie de données concernant les coccidies et leurs distributions au Canada, des échantillons fécaux provenant de 39 troupeaux de dindes, représentants 27 fermes commerciales [ON (*n* = 20), SK (*n* = 2), BC (*n* = 3), AB (*n* = 1), NS (*n* = 1)] étaient cribler pour la coccidie. L'identification de toutes les espèces d'*Eimeria* trouvées dans chaque échantillon était accomplie en utilisant une PCR nichée pour cibler la sous-unité I mitochondriale du cytochrome *C* oxydase. La plupart des échantillons (33/39) était positif pour l'*Eimeria* avec six espèces d'*Eimeria* identifiées par la PCR nichée (1 à 6 espèces/échantillon, moyenne 3,2); quatre échantillons (4/39, > 10 % d'échantillons) contenaient toutes les six espèces. Les espèces d'*Eimeria* sont communes et sont largement distribuées dans les troupeaux de dindes commerciaux canadiens. Les dindes élevées en utilisant des anticoccidiens en additifs alimentaire ou vaccinées avec des vaccins vivants pour la coccidie avaient une diversité d'espèces d'*Eimeria* similaire entre les troupeaux individuels. Ces observations préliminaires indiquent que la coccidie demeure toujours une préocculation pour les éleveurs de dindons.

(Traduit par Alex Léveillé et Lisa Gordon)

Can Vet J 2019;60:153–159

Introduction

There are 6 well-described *Eimeria* spp. that have been characterized through molecular genotyping methods; additional *Eimeria* spp. from turkeys have been named but their taxonomic validity remains uncertain. The *Eimeria* spp. widely accepted to be valid are: *E. adenoeides, E. dispersa, E. gallopavonis, E. innocua, E. meleagrimitis,* and *E. meleagridis.* Few species are believed to cause clinical coccidiosis in turkeys and information on the degree of pathogenicity for certain species is lacking (1,2). Although over 183 million kg of meat turkeys are produced annually in Canada (3), information on the distribution and identities of *Eimeria* spp. infecting turkeys in Canada has not been examined systematically. Identifying species of *Eimeria* based on morphological features alone has been shown to be unreliable because of the significant overlap in shapes and sizes of oocysts (4,5). Chapman (6) highlighted the importance of combining phenotypic characteristics as outlined by Joyner (7), such as site of development, characteristic lesions, cross-immunity, and pathogenicity, with molecular methods including DNA extraction and polymerase chain reaction (PCR) (8). Understanding the distribution and diversity of *Eimeria* spp. within Canadian turkey operations is essential for making rational decisions regarding the economic and health importance of

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these parasites and the need for their inclusion in live coccidiosis vaccines for use in turkeys.

In the present study, field samples were solicited from geographically distant locations across Canada in order to determine the number of oocysts shed in commercial turkey flocks. The diversity of *Eimeria* spp. parasites found in positive samples was further characterized using a modification of a species-specific, nested PCR assay (8).

Materials and methods

Obtaining samples and detecting *Eimeria* oocysts

A detailed letter was sent to turkey farmers across Canada requesting turkey fecal samples. Farmers were sent specific instructions outlining how the fecal samples were to be collected as well as all required collection materials and packaging for shipment to the University of Guelph. The samples were collected from the top of the litter and the freshest of droppings were to be collected. The samples were mixed with a 1:50 diluted bleach solution (provided) and sent overnight (at room temperature). Upon arrival samples were stored at 4°C until processing. Each participating farmer was asked to identify the coccidiosis control program being used (either live vaccination or in-feed anticoccidial medication) for each submitted sample; no further information regarding anticoccidial use or management was requested or obtained.

Initial detection of oocysts was made using light microscopy directly from the sample. If negative, 5 g of the original sample was mixed to homogeneity with saturated NaCl (aqueous) to a total volume of 50 mL; an aliquot of the homogenate was loaded into a McMaster counting chamber. The chamber was examined for the presence/absence of oocysts and recorded as oocyst-positive or oocyst-negative. No further processing was done on oocyst-negative samples with the exception of 2 samples that were included in the DNA extraction procedure outlined below as oocyst-negative control DNA.

Oocyst-positive fecal samples were processed for sporulation by mixing 30 g fecal sample with approximately 60 mL 2.5% potassium dichromate (w/v aqueous) and passing the homogenate through a sieve with 1 mm^2 openings. The filtrate ($<$ 100 mL) was transferred to a 250-mL Erlenmeyer flask capped with aluminum foil perforated to permit air exchange and then placed on a rotary platform shaker operating at ~100 rpm at room temperature for ~5 d to permit sporulation of viable oocysts. Following sporulation, the filtrate of sporulated/unsporulated oocysts mixed with fine fecal debris in 2.5% potassium dichromate was the source of material for DNA extraction.

DNA extraction

The DNA was extracted from all oocyst-positive samples following crude filtration and sporulation. Briefly, post-sporulation oocysts and fecal debris suspended in 2.5% potassium dichromate (w/v aqueous) were collected by centrifugation (1500 × *g* for 10 min). The supernatant was decanted from the pelleted oocysts and debris; a 2× volume of 0.9% saline (0.9% NaCl, w/v aqueous) was added to the pelleted material and mixed to homogeneity. The resulting washed, sporulated oocysts mixed with fine fecal debris were held at 4°C until DNA isolation.

The DNA was isolated using DNAzol (Life Technologies, Burlington, Ontario) following the manufacturer's instructions modified by the addition of 0.5 mm glass beads (Ferro Micro beads; Cataphote Division, Jackson, Mississippi, USA) to improve oocyst disruption as described previously (8). A sample (~1.5 mL) of the washed, sporulated oocysts mixed with fine fecal debris was pelleted by centrifugation (1200 × *g* for 2 min). After decanting and discarding the supernatant, 100 μL DNAzol reagent was added to the pellet and mixed, and approximately 0.3 g of 0.5 mm glass beads was added until a few dry beads were at the surface of the sample. This lysis mixture was processed in a horizontal bead beater fitted with a rack holder for 1.5 mL centrifuge tubes (Mickle Disintegrator l; Mickle Laboratory Engineering, Gomshall, Surrey, UK) for 60 s. Oocyst breakage was confirmed microscopically, and additional rounds of disruption were used until most of the oocysts had been lysed. Once sufficient oocyst breakage was confirmed microscopically, an additional 900 μL of DNAzol was added to the sample plus beads and the entire tube rotated at room temperature for at least 1 h and up to 14 h (overnight) to ensure complete disruption of the sample. After this process, the sample was centrifuged at 13 000 × *g* for 15 min at 4°C; the supernatant was transferred to a new 1.5 mL centrifuge tube to remove insoluble debris and glass beads; the remainder of the DNAzol extraction procedure was completed as described by the manufacturer. At the conclusion of the isolation protocol, the pelleted DNA was dissolved in 100 μL of EB Buffer provided in the QIAquick PCR purification kit (QIAGEN, Venlo, The Netherlands). Half (50 μL) of each sample was further purified using QIAquick column purification following the manufacturer's instructions. The DNA concentration was estimated spectrophotometrically both before and after column purification using a NanoDrop 2000 instrument (NanoDrop, Wilmington, Delaware, USA).

Nested PCR assay for *Eimeria* species identification

Primary PCR. A PCR-based assay based on Hafeez et al (8) was used to identify the various *Eimeria* spp. in each DNA sample. A nested PCR approach was taken to improve the sensitivity of this PCR-based assay for low abundance *Eimeria* spp. present in a mixed sample. In the first round of PCR, genus-specific primers (COI_UNI_199F — 5′-ATGATYTT CTTTGTAGTTATGCC-3′; mtRNA20_UNI_R — 5′-GTAT GGATTTCACGGTCAA-3′) that amplify a 1272 base pair (bp) fragment spanning the region from nucleotide 199 of the COI coding region to 27 nucleotides (nt) past its end were used. This covers nearly the complete cytochrome *C* oxidase subunit I region of the mitochondrial genomes of all *Eimeria* spp. Each primary PCR tube contained 500 nM of each primer, 50 mM $MgCl_2$, 1 mM dNTPs, 1× PCR buffer and 0.4 U Platinum *Taq* polymerase (Life Technologies). For the primary PCR, samples were run at 95°C for 180 s, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s and extension at 72°C for 75 s, followed by a final extension at 72°C for 5 min. Using a portion from each tube, PCR products

Table 1. PCR annealing temperatures used with the *Eimeria* species-specific PCR primers.

Species	Primer name	Annealing temperature of Hafeez et al (8)	Revised annealing temperature	Amplicon size (base pairs)
Eimeria adenoeides	E.ad.CO1_427F E.ad.CO1_1186R	62°C	64°C	713
Eimeria dispersa	E.disp.CO1_577F E.disp.CO1_1028R	55°C	59°C	451
Eimeria gallopavonis	E.gal.CO1_292F E.gal.CO1_1153R	62°C	60°C	861
Eimeria meleagridis	E.md.CO1_431F E.md.CO1_1443R	58°C	55°C	1012
Eimeria meleagrimitis	E.mel.CO1_474F E.mel.CO1_1028R	52°C	62°C	554
Eimeria innocua	E.inn.COI.396F E.inn.COI.604R	50°C	59°C	209

were electrophoresed on a 1.5% agarose submarine gel in Tris-Acetate-EDTA (TAE) buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA) at ~95 V for ~30 min. The resulting gel was stained with ethidium bromide and the product size was estimated by comparison with a 1-Kb Plus DNA Ladder (Bio Basic, Mississauga, Ontario) visualized using UV transillumination. If a primary amplicon was not visualized, a second PCR run was completed on the sample using an annealing temperature of 54°C instead of 58°C.

After electrophoresis, the intensity of each positive band was compared to the 1500 bp band of the DNA ladder to estimate amplicon abundance. If the band was estimated at ≤ 250 ng, a 1:100 dilution of the primary PCR tube contents was made using nuclease-free water; if the band was estimated to be > 250 ng, a 1:1000 dilution was made using nuclease-free water. A 2.5-μL aliquot of the appropriately diluted primary reaction solution was then used as template for each subsequent species-specific PCR reaction.

Secondary PCR. The species-specific PCR-based assay using diluted primary PCR amplicons (as described) as templates was completed as described by Hafeez et al (8) with minor modifications to the annealing conditions for each primer pair (Table 1).

Animals

Turkey poults (hens) used to propagate some field samples were received as a donation from Hendrix Genetics Hybrid Turkeys (Kitchener, Ontario). All studies were performed in the CAF (Central Animal Facilities) Isolation Unit at the University of Guelph, Guelph, Ontario, in compliance with the Canadian Council on Animal Care's *"Guide to the Care and Use of Experimental Animals,"* 2nd edition (2017; www.ccac.ca). All experiments were approved by the University of Guelph's Animal Care Committee in compliance with all institutional and national guidelines. All birds were provided feed and water *ad libitum.*

Propagation for samples negative on PCR assay

If an oocyst-positive sample tested negative in the primary PCR assay, oocysts in the sample were propagated through birds to provide fresh oocysts for characterization. Coccidia-free turkey poults (2 to 3 per cage) were inoculated with a small number of oocysts (< 2000 per bird) suspended in 1 mL 0.9% saline from a single PCR-negative sample *via* oral gavage. Inoculations *via* oral gavage were accomplished using a 1-mL Luer Slip tuberculin syringe without a needle or feeding tube fitted. Oocysts collected from such propagations were sporulated, isolated, and had DNA extracted as previously described. The DNAs isolated from such propagated samples were then used as templates for primary PCR in the same manner as DNA isolated directly from a field sample. Samples that were propagated *in vivo* before species-specific PCR were designated "PS" (i.e., Passaged Sample) whereas oocysts isolated directly from a submitted sample were designated "S" (i.e., Sample).

Results

Sample locations

A total of 39 fecal samples representing unique flocks or barns located on 27 farms were obtained from farmers who collected and sent samples to the laboratory from various locations across Canada (Table 2). Of the 27 farms, 20 farms were from numerous locations across Ontario, 2 farms were from Saskatchewan (Farms 17 and 27), 3 farms were from British Columbia (Farms 11, 12, 13), 1 farm was from Alberta (Farm 18), and 1 from Nova Scotia (Farm 14). Live coccidiosis vaccination was being used in almost half of the sampled flocks (18 samples) and anticoccidial medications were being used in the remaining flocks (21 samples).

Geographic distribution and diversity of *Eimeria* species

Oocysts of various *Eimeria* spp. were detected microscopically in 33 of 39 samples (85% prevalence) and oocyst-positive samples were recorded in samples from all provinces.

Primary and subsequent secondary nested PCR assays were performed on oocyst-positive samples as outlined in Table 2. Of the 33 oocyst-positive samples, 26 samples were positive following the primary PCR reaction and the primary product could be used for secondary nested species-specific PCR reactions.

Table 2. Summary of farm, fecal sample number, and province for samples received. Oocyst detection, polymerase chain reaction (PCR) result, and *Eimeria* species present within each turkey fecal sample are summarized.

Farm ID	Samples[a]	Coccidiosis control in use[b]	Province	Oocyst presence	Primary PCR (+/−)	Secondary species-specific PCR (+/−)[c]					
						AD	MEL	DISP	GALL	INN	MD
F1	PS1	M	ON	+	+	+	+	−	+	−	−
F1	S2	M	ON	+	−	n/a	n/a	n/a	n/a	n/a	n/a
F1	S3	M	ON	+	−	n/a	n/a	n/a	n/a	n/a	n/a
F2	PS1	V	ON	+	+	+	−	−	+	−	−
F2	PS2	V	ON	+	+	−	−	−	−	−	+
F3	PS1	V	ON	+	+	−	+	−	−	−	+
F3	PS2	V	ON	+	+	−	+	−	−	−	+
F4	PS1	M	ON	+	+	+	+	−	+	−	+
F4	S2	M	ON	+	−	n/a	n/a	n/a	n/a	n/a	n/a
F4	S3	M	ON	+	+	+	+	−	+	−	−
F4	S4	M	ON	+	+	+	+	−	+	−	+
F5	PS1	M	ON	+	+	−	+	−	+	−	−
F6	PS1	V	ON	+	+	−	+	+	+	−	−
F7	S1	M	ON	+	+	−	+	−	−	−	−
F8	PS1	M	ON	+	+	−	+	−	−	−	−
F9	PS1	V	ON	+	+	−	+	−	−	−	+
F10	S1	M	ON	+	+	+	+	−	+	−	+
F11	PS1	M	BC	+	+	+	+	−	−	+	+
F12	PS1	M	BC	+[d]	−	n/a	n/a	n/a	n/a	n/a	n/a
F13	PS1	M	BC	+	+	−	+	−	+	−	+
F14	S1	M	NS	+	+	+	+	−	−	−	+
F15	S1	V	ON	+	−	n/a	n/a	n/a	n/a	n/a	n/a
F16	PS1	V	ON	+	+	−	+	+	+	−	−
F17	S1	M	SK	+	+	−	+	−	−	−	−
F18	S1	V	AB	+	+	+	−	−	−	−	−
F19	S1	V	ON	+	−	n/a	n/a	n/a	n/a	n/a	n/a
F20	S1	V	ON	+	+	−	+	+	+	+	+
F20	S2	V	ON	+	−	n/a	n/a	n/a	n/a	n/a	n/a
F20	S3	V	ON	+	+	−	+	+	+	+	+
F21	S1	V	ON	+	+	+	+	+	+	+	+
F21	S2	V	ON	+	+	+	+	+	+	+	+
F21	S3	V	ON	+	+	+	+	+	+	+	+
F21	S4	V	ON	+	+	+	+	+	+	+	+
F22	S1	M	ON	−	n/a	n/a	n/a	n/a	n/a	n/a	n/a
F23	S1	V	ON	−	n/a	n/a	n/a	n/a	n/a	n/a	n/a
F24	S1	M	ON	−	n/a	n/a	n/a	n/a	n/a	n/a	n/a
F25	S1	M	ON	−	n/a	n/a	n/a	n/a	n/a	n/a	n/a
F26	S1	M	ON	−	n/a	n/a	n/a	n/a	n/a	n/a	n/a
F27	S1	M	SK	−	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Total positive				33	26	13	23	8	16	7	16

[a] Samples designated "S" were identified as oocyst-positive or negative and DNA extraction for PCR was done on oocyst-positive samples; samples designated "PS" had detectable oocysts in the original sample but primary PCR was negative. 'PS' samples were passaged in coccidia-free poults before DNA extraction and primary PCR.
[b] V — vaccinated; M — medicated with anticoccidial drugs.
[c] Species-specific PCR test for AD — *Eimeria adenoeides;* DISP — *Eimeria dispersa;* GALL — *Eimeria gallopavonis;* INN — *Eimeria innocua*; MD — *Eimeria meleagridis;* MEL — *Eimeria meleagrimitis.*
[d] Fecal sample was oocyst-positive when received but when the oocysts were passaged through poults, the poults did not shed oocysts.
n/a — Not available.

There was considerable diversity in the number and specific species detected in the samples using the nested PCR assay; 1 to 6 different *Eimeria* spp. were detected in oocyst-positive samples. One location from Ontario had all known *Eimeria* spp. in turkeys (i.e., *E. adenoeides, E. dispersa, E. gallopavonis, E. innocua, E. meleagridis,* and *E. meleagrimitis*) present in each of 4 submitted samples. The 1 positive sample received from 1 farm in Nova Scotia contained *E. adenoeides, E. meleagridis,* and *E. meleagrimitis.* The 1 sample received from the single sampled farm in Alberta contained only *E. adenoeides.* Of the 2 samples from 2 farms in Saskatchewan, only 1 sample was positive and it contained *E. meleagrimitis.* Of the 3 samples from 3 different farms in British Columbia, 2 samples contained 3 or 4 *Eimeria* spp. each representing 5 unique *Eimeria* spp.; only *E. dispersa* was not detected in the BC samples.

Overall, the species diversity (Table 3) of *Eimeria* present in samples coming from flocks using live coccidiosis vaccination (14 PCR-positive samples with 3.6 species/sample) was higher than the species diversity found in samples from medicated flocks (12 PCR-positive samples with 2.8 species/sample). However, exclusion of the 2 *Eimeria* spp. expected to be present in all flocks administered the live coccidiosis vaccine (i.e., *E. adenoeides* and *E. meleagrimitis*) resulted in remarkably similar mean parasite species diversity in vaccinated and medicated flocks at 2.4 and 2.8 *Eimeria* species/sample, respectively.

Discussion

Prior to the present study, the diversity of *Eimeria* spp. infecting turkeys on Canadian commercial farms was largely unknown because suitable fecal sampling and analyses from various

Table 3. Summary of oocyst positive samples from each province, *Eimeria* species found in each province, number of samples from vaccinated and medicated flocks and average species diversity per province.

Location	Oocyst positive	PCR positive	AD	DISP	GALL	INN	MD	MEL	Average species diversity (species/sample)
AB	1	1	1	0	0	0	0	0	1
BC	3	2	1	0	1	1	2	2	3.5
NS	1	1	1	0	0	0	1	1	3
ON	27	21	10	8	15	6	13	19	3.4
SK	1	1	0	0	0	0	0	1	1
All of Canada	33	26	13	8	16	7	16	23	3.2

Summary of PCR positive samples	PCR + ve	Average species diversity
Vaccinated flocks (Immucox-T)	3.6	
Vaccinated flocks (excluding Immucox-T constituents)[a]	14	2.4
Medicated flocks (Anticoccidials)	12	2.8

[a] Immucox-T contains *Eimeria adenoeides* and *Eimeria meleagrimitis.*
AB — Alberta; BC — British Columbia; NS — Nova Scotia; ON — Ontario; SK — Saskatchewan.
AD — *Eimeria adenoeides;* DISP — *Eimeria dispersa;* GALL — *Eimeria gallopavonis;* INN — *Eimeria innocua;* MD — *Eimeria meleagridis;* MEL — *Eimeria meleagrimitis.*

geographic locales had not been attempted systematically. With few exceptions (9), there was a paucity of information regarding *Eimeria* spp. on farms in Canada. Furthermore, the available data were based universally on morphological diagnoses. Molecular and biological data (2,5,8,10–14) have become available for turkey *Eimeria* species only recently, permitting detailed studies that were previously challenging to undertake because of the difficulty in differentiating these parasites in field samples (1).

The preliminary prevalence data obtained in this study suggest that the 6 generally recognized *Eimeria* spp. capable of infecting turkeys are distributed widely within Canadian commercial turkey flocks. At least some *Eimeria* spp. are common, abundant, and widespread in these flocks. The composition and diversity of parasites in individual samples varied widely and, despite the relative paucity of samples from outside of Ontario in the survey, a diverse parasite fauna was found across the country. Previous reports based on morphological identification of *Eimeria* spp. from the USA (15–17) and the UK (18,19) had suggested that *Eimeria* spp. of turkeys were common and distributed widely in commercial turkey flocks. Direct comparisons between previous studies and the present work are complicated by the limitations of such morphological identifications (5). Nonetheless, the 85% (33/39) prevalence of the highly pathogenic parasite, *E. meleagrimitis,* in US samples detected by Edgar (16) was remarkably similar to the 88% prevalence (23/26) of the same parasite in the present study.

Most samples (85%) herein had detectable oocysts and the number of detectable *Eimeria* spp. in the positive samples ranged from 1 to 6 with an average of 3.2 species/sample detected among all PCR-positive samples. Flocks that were vaccinated had more species diversity (average of 3.6 *Eimeria* species/sample) compared to samples from medicated flocks but this greater species diversity was largely the result of including the vaccine constituents found in Immucox-T in the species count. The species diversity was essentially identical after correcting for the species found in the vaccine. Generally, oocyst-positive samples obtained from vaccinated and medicated flocks that were successfully amplified using the nested PCR assay had 2 to 3 *Eimeria* spp. present.

The diversity of *Eimeria* spp. circulating in Canadian commercial turkey flocks was remarkably similar to the prevalence and diversity of *Eimeria* spp. found in hunter-harvested wild turkeys in Ontario. As in the commercial flocks, most of the sampled hunter-harvested wild turkeys were infected (77% *Eimeria* oocyst-positive, n = 107) (20). Earlier investigations using morphometric data to differentiate parasites (9,21–23) had shown that *Eimeria* spp. were common and relatively diverse in wild turkeys in the southern USA; more recently, MacDonald et al (20) used molecular methods similar to those used in the present work to demonstrate that *Eimeria* spp. were common and diverse in Ontario wild turkeys. Interestingly, 4 of the 6 species found within commercial flocks in the present study (i.e., all except *E. dispersa* and *E. innocua*) were also found in wild turkeys in Ontario, Canada, with an average of 2.6 *Eimeria* species per oocyst-positive bird. In the present study, the 26 oocyst-positive samples that were PCR positive had similar species diversity (2.8 and 2.4 species/samples for medicated and vaccinated flocks, respectively) to the hunter-harvested wild turkeys even though additional *Eimeria* spp. were detected in the commercial birds. It is probable that all *Eimeria* spp. would be detected in both wild and domestic turkeys across their distributions in Canada given sufficient sampling effort; it is likely that any geographic restriction of particular *Eimeria* spp. (e.g., *E. dispersa* found only in Ontario samples) reflects the limited sampling more than restricted geographic range.

With similar prevalence and diversity of *Eimeria* spp., wild turkeys may be potential reservoirs for *Eimeria* spp. infecting commercial poultry; there is no physiological or other impediment against *Eimeria* spp. that infect commercial turkeys infecting wild turkeys or *vice versa* (1,20). Commercial turkey farms are widespread across Canada and may be in close association with wild turkey habitats that may provide opportunity for transfer of pathogens from wild to domestic flocks and *vice versa.* The opportunity for such cross infections in Canada was negligible until the reintroduction of wild turkeys during the 1980's

was undertaken to reverse the extirpation (local extinction) of *Meleagris gallopavo* during the early 20th century through widespread hunting and loss of habitat. In 1984, captive-bred wild turkeys from the United States were released to initiate their re-establishment (24); that has resulted in a flourishing wild turkey population in Canada (Eastern and Western) (25). Wild turkeys have been shown to shed *Eimeria* spp. oocysts into the environment in Ontario (20) and these parasites may be carried into commercial operations. *Eimeria* spp. apparently overwinter within infected wild turkeys or within the environment suggesting that they may have adapted to the harsh local climatic conditions. Such hardy parasites may be important reservoirs of infection for turkey barns that are typically "all-in-all-out" operations; brooder barns, in particular, are typically cleaned thoroughly between each flock (26). Conversely, commercial turkey operations must compost, dispose of, or spread their manure depending on the farm's nutrient management plan. This spreading of manure into the environment may provide a source of *Eimeria* spp. for wild turkeys and an alternate way that commercial turkeys could be infecting wild populations. With this potential cycle of infection between wild and domestic animals and increased potential of spreading infection within the higher stocking density of commercial farms, eliminating *Eimeria* spp. from commercial or wild populations seems challenging. As a result, preventing this condition may be the best option to reduce the economic burden on the industry.

Although many *Eimeria* spp. of turkeys are considered only mildly pathogenic (1,2,10) there may be more numerous pathogenic species than previously assumed (i.e., not only *E. adenoeides* and *E. meleagrimitis*). The diversity of species identified across Canada begs the question as to whether or not the existing Immucox-T vaccine can protect against all pathogenic *Eimeria* spp. present in a turkey flock. A recent well-controlled *in vivo* cross-species challenge study (27) confirmed that no significant adaptive cross-immunity was detected among any of the 6 *Eimeria* species found in turkeys despite robust protection against homologous challenge. However, some limited, non-specific (innate) protection was conferred by 1 *Eimeria* sp. against other *Eimeria* spp. residing in the same region of the intestinal tract (27). Cross-challenged birds immunized with 1 "cecal" species challenged with a different cecal species (i.e., "same-zone" challenge) had numerically decreased oocyst output compared to birds challenged similarly but immunized with an *Eimeria* sp. inhabiting a different region of the intestine (i.e., "cross-zone" challenge). For example, a bird immunized with *E. adenoeides* (a cecal species) challenged with *E. meleagridis* or *E. gallopavonis* (also cecal species), had a 48% decrease in oocyst output compared with cross-zone challenges (i.e., an "intestinal" species challenged with a cecal species) (27). Consequently, it was suggested (27) that Immucox-T vaccine may provide limited, non-specific protection against *Eimeria* spp. that inhabit the same regions of the intestinal tract as the parasites in the vaccine. The 2 constitutive *Eimeria* spp. in the vaccine that inhabit both the intestinal and cecal regions should provide robust, immune-mediated protection against homologous challenge (i.e., *E. meleagrimitis* and *E. adenoeides* vaccine constituents) with proper vaccine management and at least limited, non-specific protection against all other *Eimeria* spp. that infect turkeys.

The present study identified common *Eimeria* spp. found on a limited number of commercial farms in Canada sampled in a consistent manner and analyzed using newly available molecular diagnostic methods (8) for determining the prevalence of the various *Eimeria* spp. However, further research is needed to gain a more detailed understanding of the geographic and seasonal distribution of *Eimeria* spp. in more regions of Canada. Establishing the diversity and distribution of the various *Eimeria* spp. in Canadian commercial flocks may be useful for managing the use of the single licensed live coccidiosis vaccine currently registered for use in turkey (Immucox-T containing *E. adenoeides* and *E. meleagrimitis*), as well as prophylactic anticoccidial use.

Acknowledgments

We thank Julia Whale and Julie Cobean for their technical assistance and support. Perryn Kruth, Alex Léveillé, Evelin Rejman, and Ryan Snyder are thanked for their contributions. The French translation of the fecal sample request letter to turkey farmers in Quebec and abstract were provided by Alex Léveillé and Lisa Gordon. The staff of the CAF Isolation Unit, University of Guelph, are thanked for their ongoing superior care and assistance with experimental animals. This research was funded through grants from the Natural Sciences and Engineering Research Council of Canada (NSERC — Discovery Grant #400566) and the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA — Tier II #200331) to JRB. RKI was supported, in part, by an Ontario Veterinary College Scholarship (MSc). The donation of turkey poults by Hendrix Genetics, Kitchener Ontario, Canada is acknowledged with thanks. CVJ

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